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(54) Title: METHOD OF PREVENTING AND TREATING INSULIN DEPENDENT DIABETES MELLITUS

(57) Abstract

A method for the treatment of Insulin Dependent Diabetes Mellitus (IDDM) is disclosed. This method comprises administering to a human who is suffering from said IDDM, an amount of Interleukin-1 Receptor Antagonist Protein (IRAP) effective to reduce the severity of said IDDM.

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METHOD OF PREVENTING AND TREATING INSULIN DEPENDENT DIABETES MELLITUS

FIELD OF THE INVENTION

The present invention relates to the treatment of insulin dependent diabetes mellitus (IDDM) using Interleukin 1 Receptor Antagonist Protein (IRAP).

BACKGROUND OF THE INVENTION

Diabetes mellitus (DM) is characterized by a broad array of physiologic and anatomic abnormalities, but its most notable feature is disturbed glucose metabolism, resulting in inappropriate hyperglycemia. In fact, diagnosis is usually based on basal, postprandial, or post-glucose load measurements of blood or plasma glucose-levels. Estimates from the National Health Interview Survey indicate that about 2.4 percent of the United States population, or 5.5 million people, consider themselves to be diabetic.

DM has no distinct etiology, pathogenesis, invariable set of clinical findings, specific laboratory tests, or definitive and curative therapy, although it is nearly always associated with fasting hyperglycemia and decreased glucose tolerance. The complete clinical syndrome of DM involves hyperglycemia, large-vessel disease, microvascular disease (retina and kidney) and neuropathy.

Diabetic conditions are generally divided into two categories: insulin-dependent diabetes mellitus (IDDM or Type I) and non-insulin-dependent diabetes mellitus (NIDDM or Type II). Patients who depend on insulin for the prevention of ketoacidosis have IDDM. IDDM most often develops in childhood or adolescence, so this form of the disease was previously termed juvenile-onset diabetes; other names for IDDM are ketosis- or acidosis-prone and Type I diabetes. IDDM patients are literally dependent on exogenous insulin to prevent ketoacidosis and death. This type of diabetes is associated with certain histocompatibility antigens (HLA) on chromosome 6, with autoimmunity directed against the islet, and possibly with a predisposition to viral infections. Viruses of several types are some of the environmental agents that may induce IDDM in genetically susceptible persons, perhaps involving cell-mediated immune mechanisms.

DM results in suppressed insulin secretion. The lack of insulin causes elevated blood glucose levels referred to as hyperglycemia. The classification of a patient as IDDM or NIDDM is often based upon the severity of insulin secretion impairment experienced. Other physiological problems encountered by patients with DM include: large vessel disease such as increased atherosclerosis, a greater risk of cardiovascular death, and peripheral vascular disease; microvascular disease such as abnormality in the thickness of the basal lamina of capillaries, frequently in the retina and retina glomeruli; and, segmental injury to nerve cells. In addition, patients suffering DM, particularly IDDM, face the life threatening risks of

ketoacidosis.

The clinical onset of IDDM seems to be preceeded for many years by the appearance of islet cell antibodies (ICA). High titers of ICA in first degree relatives of diabetic patients, especially when associated with an impaired first phase insulin release, is almost always followed by overt IDDM. The earliest symptom of elevated blood glucose is polyuria from the osmotic diuretic effect of glucose. Continued hyperglycemia and glucosuria may lead to thirst, hunger and weight loss. Glucosuria is also associated with an increased incidence of monilial vaginitis and itching. It is uncertain whether the incidence of other infections (e.g., pyelonephritis, cystitis) is increased as a direct result of hyperglycemia. Accelerated fat catabolism in the untreated insulin-dependent patient produces ketoacidosis leading to anorexia, nausea, vomiting, air hunger, and, if untreated, coma and death. Clinical onset tends to be abrupt in children and insidious in older patients.

The symptoms and signs of large-vessel atherosclerosis in the diabetic are the same as in nondiabetic patients. The symptoms and signs of microvascular disease are those of renal failure if the glomerular capillaries are involved, or visual loss if the retinal capillaries are affected. Proteinuria usually is the first indication of nephropathy, and it may reach nephrotic levels. The greater the proteinuria, the more rapid is the development of renal failure. Renal failure is seen in 50% of IDDM patients after 20 to 30 yr of diabetes. Diabetic retinopathy is usually first detected 5 yr or more after the diagnosis of DM is made and is present to some degree by 10 yr in 50% of patients.

In treating DM, the primary objective is to achieve the patient's optimal health and nutrition. An integrated index of long-term blood glucose control is now available through the use of stable glucosylated Hb determinations. Normally about 7% of HbA molecules are modified during erythrocyte synthesis. Since the half-life of this cell and its Hb is 60 days, the percent of stable glucosylated Hb reflects the mean blood glucose concentration over the preceding 2 mo. With the removal of the labile glucosylated Hb fraction prior to assay, the final result is not significantly influenced by glucose fluctuations. Determinations of glucosylated Hb are helpful in judging the degree of chronic glucose control in both IDDM and NIDDM patients and in judging efficacy of changes in therapy.

The objectives of the treatment of diabetes are (1) to avoid ketoacidosis, (2) to control symptoms resulting from hyperglycemia and glucosuria, and (3) to prevent the micro and macrovascular complications of diabetes. Through the use of self blood glucose monitoring (SBGM) techniques involving reagent test strips with or without a reflectance meter, more normal blood and urine glucose levels have become a realistic goal for many patients with diabetes. Several sulfonylureas that can lower the blood glucose level when given orally may be used to treat selected patients. For IDDM patients, exogenous insulin must be administered

to maintain proper blood glucose levels.

The present invention provides a method of treating IDDM comprising administration of an effective amount of Interleukin-1 Receptor Antagonist Protein (IRAP). The cytokine interleukin-1 (IL-1) may have an important role in the autoimmune mediated damage of pancreatic B-cells found in patients suffering from IDDM. IRAP, a specific blocker of the IL-1 receptor, prevents the deleterious actions of recombinant IL-1 on insulin-producing cells. It is believed that by preventing the suppressive actions of IL-1, the administration of IRAP will protect the pancreatic B-cells from being damaged and, therefore, prevent the onset of pathophysiology associated with IDDM. Thus, patients susceptible to IDDM will not require exogenous insulin since they will not experience impaired insulin secretion. They will likewise be spared of all other debilitations related to IDDM.

INFORMATION DISCLOSURE

PCT International Application Number PCT/US88/02819 published 9 March 1989 discloses an Interleukin-1 inhibitor purified from urine which is characterized by its inhibitory activity as measured by several assays. The IL-1 inhibitor disclosed has a pI of 4.7.

PCT International Application Number PCT/US89/02275 published 30 November 1989 discloses an Interleukin-1 inhibitor purified from cultures human monocytes. Furthermore, an inhibitor gene is disclosed and a recombinant inhibitor described.

Liao et al., J. Exp. Med. 159:126-136 (1984) teach the identification of an IL-1 inhibitor found in urine from febrile patients. The IL-1 inhibitor disclosed by Liao et al. has a molecular mass of between 20-40 kdal. Liao et al. note that the evidence suggests that the molecule is a protein or a glycoprotein but that the evidence is insufficient to support such a statement without additional information.

Arend et al., J. Immunol. 134:6 (1985) teach an inhibitor of IL-1 interaction with chondrocytes or thymocytes which is produced by human monocytes cultured on adherent immune complexes or antibodies. The molecular mass of this factor is approximately 22 kdal.

Seckinger and Dayer, Ann. Inst. Pasteur Immunol., 138 (3):486-488 (1987) discuss various IL-1 inhibitors. Several inhibitors are disclosed which have been found in urine from highly febrile patients. Among the inhibitors are those having molecular masses of 30-35 kdal, 85 kdal, and 18-25 kdal. The IL inhibitor of molecular mass 18-25 kdal is an immuno-suppressant glycoprotein isolated from urine of pregnant women. Other IL-1 inhibitors disclosed in Seckinger and Dayer are the 22 kdal molecule reported by Arend, et al. which is derived from human monocytes stimulated by adherent immune complexes. A 95 kdal molecule derived from human monocytes stimulated by cytomegalovirus is also disclosed. Additionally, an IL-1 inhibitor with a molecular mass of about 95 kdal derived from human macrophages exposed to influenza and syncytial virus and from human virus-infected B cells is

reported.

Hannum, C. H. et al., Nature 343:336-340, January 1990, disclose three Interleukin-1 inhibitors produced by human monocytes. Partial protein sequence data indicates the three isoforms of a single protein.

Eisenberg,, S. P. et al., Nature 343:341-346, January 1990, disclose cDNA clone for an Interleukin-1 receptor antagonist produced by human monocytes induced with adherent IgG. The cDNA disclosed is cloned to E. Coli where it is expressed to yield Interleukin-1 inhibitor.

Carter, D. B., et al., Nature 344:633-638, April 1990, disclose a cDNA clone for Interleukin-1 Receptor Antagonist Protein IRAP isolated from U937 cells.

Eizirik, D.L. et al., Diabetes, Vol. 37, No. 7, pp. 916-919, (July 1988) disclose that the function of rat pancreatic islets is effected by exposure to IL-1 for 48 hr and indicate that IL-1 is cytotoxic to islet B cells. The islets were examined immediately after Il-1 exposure or after an additional 6-day culture period without IL-1. Results indicate that IL-1 totally inhibited glucose stimulated insulin release, partially inhibited glucose oxidation, and induced a decrease in islet DNA content when evaluated immediately after IL-1 exposure. After a 6-day period of culture without IL-1, insulin secretory response to glucose and the glucose oxidation rates were completely restored but there remained a reduced islet DNA content. However, when cultured in the absence of IL-1 surviving B-cells were able to completely recover their functionality after a period of inhibited function.

Sandler, S. et al., Endocrinology, Vol 124. No. 3, pp. 1492-1500 (1989) disclose that IL-1 induced inhibition of insulin secretion in rat pancreatic islet cells is not related to mechanisms by which alloxan or streptozotocin impair B-cell function.

Eizirik D. L., and Sandler, S., Diabetologia, 32:769-773 (1989) disclose that stimulation of insulin-release from pancreatic islet cells induced by acute exposure to human interleukin-1 is accompanied by an increase in mitochondrial oxidative events. It is disclosed that the suggested interleukin-induced inhibition of islet function mediated through impairment of oxidative metabolism is related to the same changes in substrate metabolism responsible for acute stimulatory effects of IL-1β on islet functions.

Eizirik D. et al., Endocrinology, Vol. 125. No. 2, pp. 752-759 (1989) suggests that IL-1 preferentially inhibits mitochondrial functions, especially in initial steps of the Krebs cycle.

Hammonds, Peter et al., FEBS Lett., Vol. 261, number 1, pp. 97-100 (February 1990) disclose the presence of specific high and low affinity binding sites for IL-1β in insulin-secreting B-cells.

Eizirik, D.L., et al., Endocrinology, 126:1611-1616 (1991) disclose that IL-1 induced inhibition of insulin secretion in rat pancreatic islets is mediated by activation of gene transcription and protein translation. Similar findings are reported by Eizirik, Autoimmunity,

10:107-113 (1991) in mouse pancreatic islets.

Eizirik, D.L., et al., Diabetologia, 34:445-448 (1991) shows that IRAP can protect rat and mouse pancreatic islets, and an insuliinoma cell line (RINm5F cells), against the suppressive and cytotoxic actions of IL-1.

SUMMARY OF THE INVENTION

The present invention provides a method for the treatment or prevention of insulin dependent diabetes melitis (IDDM) in mammals. This method comprises administering to a mammal, who is suffering from or is particularly susceptible to said IDDM, an amount of Interleukin-1 receptor antagonist protein (IRAP) effective to cure or prevent said IDDM.

DETAILED DESCRIPTION OF THE INVENTION

The cytokine interleukin-1β (IL-1β) may have an important role in the autoimmune mediated damage of pancreatic B-cells in insulin-dependent diabetes mellitus. In the present study we have investigated the effects of IRAP, a specific blocker of the type 1 IL-1β receptor, on the suppressive actions of recombinant IL-1β, (rIL-1β) on insulin-producing cells. Brief exposure (1-2 hr) of rat and mouse pancreatic islets to 10 ng/ml rIL-1β or rIL-1α induced a 70-80% inhibition of insulin response to glucose after 12 h. These effects were completely counteracted by coincubation with 100-1000 ng/ml IRAP. When rat islets were cultured for 48 hrs in the presence of rIL-1β (5 ng/ml) and IRAP (500 ng/ml) there was complete protection against IL-1 induced impairment in insulin release and decrease in islet insulin and DNA content. IRAP also counteracted the inhibitory effects of rIL-1β on the growth of the rat insulinoma cell line RINm5F. These data suggest that IRAP can protect insulin producing cells from the deleterious effects of rIL-1β, and that these cells possess type 1 IL-1β receptor.

The clinical outbreak of insulin-dependent diabetes mellitus (IDDM) is preceded by a chronic autoimmune assault to the pancreatic B-cells. It has been suggested that the cytokine interleukin-1 (IL-1) may be one of the main mediators of this autoimmune reaction. It has been shown that long-term in vitro exposure of rodent pancreatic islets or insulinoma cell lines, to recombinant IL-1β (rIL-1β) suppress insulin production and release, and can lead to B-cell death.

Although the ultimate mechanism of action of rIL-1β on insulin producing cells remains to be clarified, exposure to IL-1 results in activation of gene transcription and protein translation and, in the case of rat islets, progressive impairment of mitochrondial function or, in the case of mouse islets, decreased insulin mRNA and consequence decrease in proinsulin biosynthesis and release. There are data to suggest that the cytokine bind to specific surface receptors.

The complexity of the putative molecular mechanism of action IL-1β in the B-cells makes it difficult to envisage a way to protect these cells from the deleterious effects of IL-1.

Probably the most feasible approach would be to block the binding of the cytokine to the B-cell receptors. Recently an interleukin-1 receptor antagonist protein (IRAP) has been purified, cloned and expressed in E. coli, PCT/US89/02275, incorporated herein by reference. IRAP specifically blocks the type 1 IL-1 receptor, expressed in T cells and fibroblasts.

To demonstrate that IRAP is useful in treating IDDM, a series of in vitro experiments were performed that examined the effect of IRAP on the suppression of insulin releasing activity in cells exposed to IL-1. Experiments were performed on rat pancreatic islet cells, mouse pancreatic islet cells and a rat insulinoma cell line, RINm5F. In some experiments, the effects of IRAP on insulin release by cells exposed to IL-1 was evaluated. These experiments included the exposure of cells to IRAP at various times relative to the exposure of the cells to IL-1. In addition, in other experiments DNA content was measured to determine the effect of IRAP on the cell growth and/or survival of cells that produce insulin but which had been exposed to IL-1.

Insulin release experiments evaluated the effect of IRAP exposure on the suppression of insulin releasing activity caused in cells by short- and long-term exposure to IL-1. Rat pancreatic islet cells were exposed to IL-1 for one hour, two hours or 48 hours; glucose stimulated insulin release or insulin accumulation in culture medium was then measured. IRAP was added to the cells 15 minutes before one hour exposure to IL-1. IRAP was added after the first hour of IL-1 exposure in cells exposed to IL-1 for two hours. IRAP was added at the same time as IL-1 in the 48 hour exposure experiments, and the medium changed every 12 hrs with the addition of fresh IRAP and IL-1. In all three sets of experiments, cytokine containing medium was replaced after the allotted time of exposure. Glucose stimulated insulin release 12 hours after exposure to cytokine was measured in the first two time experiments. In the 48 hour experiments, the glucose stimulated insulin release was measured immediately after exposure to IL-1 and/or IRAP. In the 48 hour group, DNA and insulin content was also measured, which is discussed below. Similar insulin release experiments were performed on mouse pancreatic islet cells to evaluate the effect of IRAP exposure on the suppression of insulin releasing activity caused in cells by exposure to IL-1 for one hour.

To determine whether or not the protective effect IRAP has on IL-1 exposed islet cells is due to direct action on B cells or through non-B cell intermediates, the effects of IL-1 and IRAP on the insulinoma cell line RINm5F were tested. Experiments compared the effect of exposure of IL-1 alone, IRAP alone, and IL-1 plus IRAP on cell replication and DNA content over a period of 48 hours.

The biological activity of human recombinant IL-1β that was used was 5 U/ng, as compared with an interim international standard rIL-1β preparation (NIBSC, London, UK). The concentrations of rIL-1β that were used (5 or 10 ng//ml) have been found to functionally

suppress pancreatic islets and arrest the growth of the rat insulinoma cell line RINm5F without inducing widespread cell killing. Recombinant IRAP was prepared as described in Carter, D.B. et al., Nature, 344:633-638 (1990).

Pancreatic islets were isolated by collagenase digestion from adult male Sprague-Dawley rats bred in a local colony (Uppsala, Sweden) or from adult male NMRI mice (Anticimex, Sollentune, Sweden). The rat insulinoma cell line used was RINm5F. The rat and mouse islets were cultured free-floating in medium RPMI 1640 containing 11.1 mM glucose and supplemented with 10% (vol/vol) of donor calf serum. Growing RINm5F cells were trypsinized and subcultured in RPMI 1640 supplemented with 10% (vol/vol) fetal calf serum.

Exposure of rat and mouse islets to rIL-1β was performed for 60-120 min. in culture medium, as described above. When cells were to be exposed to IL-1 for 60 min., IRAP was always added 15-20 min. before rIL-1β. In experiments in which cells were exposed to IL-1 for 120 min., IRAP was added after the first 60 min. of IL-1 exposure. After exposure to rIL-1β and/or IRAP, the islets were washed in RPMI 1640, transferred to new culture dishes and maintained in culture medium for 12 hr without any further additions before functionally studied. In some experiments, rat islets or RINm5F cells were cultured for 48 hr in the presence of both rIL-1β and IRAP before functional studies.

Insulin release, insulin accumulation into the medium, DNA and insulin contents was determined as described in Sandler S., et al., Endocrinology 121:1424-1431 (1987). Briefly, insulin release was studied in triplicate groups of 10 islets by a first hour incubation at 1.7 mM glucose. The incubation medium, Krebs-Ringer bicarbonate buffer was supplemented with 2 mg/ml BSA and 10 mM Hepes (KRBH). After the first hour, the medium was gently removed and replaced by KRBH containing 16.7 mM glucose and the incubation continued for a second 60 min. period.

For the determination of RINm5F cells growth, 1 μCi/ml ^{3}H-thymidine was added to the culture medium during the last 1 hr of a 48 hr incubation in the presence of rIL-1β and IRAP, and thymidine incorporation measured as described in Sandler, S., et al., Immunol. Lett. 22:267-272 (1989).

One hour exposure of rat pancreatic islets to 10 ng/ml rIL-1β induced a 80% decrease in glucose-stimulated (16.7 mM) insulin release 12 hours after exposure to the cytokine (Table 1). The presence of 10 ng/ml IRAP did not modify the suppressive effects of rIL-1β, but IRAP concentrations in the range of 100 to 1000 ng/ml were able to completely block the effects of rIL-1β ($P < 0.001$ as compared to islets exposed to rIL-1β). IRAP by itself did not modify islet function. In these and the experiments described below (see Tables 1 and 2) there were no differences in the basal insulin secretion at 1.7 mM among the various control and experimental groups. The pooled values at 1.7 mM glucose of the control islets, cultured in

the absence of rIL-1β or IRAP, was 4.7 $\pm$ 0.8 ng/10 islets x 60 min. (n = 23).

In a second series of experiments islets were exposed for 2 hr to rIL-1, and IRAP (1000 ng/ml) was added to the medium just during the second hour (see second column, Table 1). After 12 hr rIL-1β induced a similar 80% decrease in insulin release, as observed in experiments in which cells were exposed to IL-1 for 60 min. However, in the experiments in which cells were exposed to IL-1 for 120 minutes, the observed decrease in insulin release was not significantly influenced by IRAP. These findings suggest that IRAP inhibit rIL-1β actions on B-cells by blocking surface receptor binding, and will not be able to counteract the effects of the cytokine once the initial binding and putative generation of intracellular second mediators has occurred.

One hour exposure of rat pancreatic islets to rIL-1α (20 ng/ml) induced a similar suppression of glucose (16.7 mM)-induced insulin release after 12 hrs as observed with rIL-1β. Thus, the control islets released 54 $\pm$ 12 ng insulin//10 islets x 60 min (n = 3, $P<0.02$). IRAP (2 μg/ml) was able to block this inhibitory effect of rIL-1α (IRAP + rIL-1α, 69 $\pm$ 6 ng insulin/10 islets x 60 min, n = 3). As described above, IRAP by itself did not interfere with the insulin release of rat pancreatic iislets (IRAP-treated islets, 61 $\pm$ 10 ng insulin/10 islets x 60 min, n = 3). Thus, IRAP can protect insulin-producing cells against both forms of IL-1 (IL-1β and IL-1α).

In all short-term experiments using rat islets there were no differences in DNA or insulin content among the different control and experimental groups. The pooled DNA and insulin content values for the control islets were 319 $\pm$ 18 ng DNA/10 islets and 751 $\pm$ 53 ng insulin/10 islets.

To evaluate the protective effects of IRAP against rIL-1β under long-term culture conditions, rat islets were incubated in the presence of rIL-1β (5 ng/ml) and IRAP for 48 hours and the medium changed every 12 hrs with the addition of fresh IRAP and rIL-1β. Culture in the presence of high concentrations of IRAP did not affect islet function, as evaluated by acute glucose-stimulated insulin release, DNA and insulin content (Table 2). rIL-1β inhibited insulin release by 90% and also induced a 35% decrease in islet insulin content and a 30% decrease in islet DNA content ($P<0.02$ or less). All these effects of rIL-1β were completely counteracted by 500 ng/ml IRAP (Table 2). These findings suggest that long-term exposure of rat pancreatic islets to rIL-1β not only suppress β-cell function, but also decrease islet cell content, as evaluated by the decreased islet DNA content. Both deleterious effects of the cytokine were prevented by blocking surface receptor binding with IRAP.

Two hour exposure of mouse pancreatic islets to rIL-1β (10 ng/ml) induced a similar suppression of glucose-induced (16.7 mM) insulin release after 12 hr as observed in the rat islets. Thus, the control islets released 52 $\pm$ 3 ng insulin/10 islets x 60 min. and the rIL-1β-

treated islets 16 ± 5 ng insulin/10 islets x 60 min (n = 4, $P<0.05$). IRAP (500 ng/ml) was able to block this inhibitory effect of rIL-1β (IRAP + rIL-1β-treated islets, 41 ± 7 ng insulin/10 islets x 60 min, n = 4, $P>0.2$ vs. controls and $P<0.05$ vs. rIL-1β-treated islets). IRAP by itself did not interfere with the insulin release of NMRI islets (IRAP-treated islets, 45 ± 3 ng insulin/10 islets x 60). Thus, mouse islet cells seem to possess similar IL-1 receptors as the rat islet cells.

The islets of Langerhans contain an heterogeneous cell population. Even considering that the prolonged preculture (5-7 days) before exposure to rIL-1β possibly eliminated most of the non-endocrine cells in the islets, it cannot be excluded that rIL-1β acted through generation of a secondary signal from non-B cells. If this is the case, the protective action of IRAP could be due to blocking the IL-1 receptor on non-B-cells. To address this issue, the effects of rIL-1β and IRAP on a insulinoma cell line, RINm5F were investigated. While rIL-1β induced a reduction of both cell replication and DNA content over a period of 48 hr in culture (Table 3), these effects were completely counteracted by IRAP ($P<0.05$ vs. rIL-1β).

Together with the previous observations that a hamster insulin-producing cell line possess specific receptors for rIL-1β (Hammonds P. et al., FEBS Lett. 261:97-100, 1990), these findings suggest that both the rIL-1β actions and the protection induced by IRAP occurs through direct interactions with B-cells, rather than via activation of other intermediary cells.

The in vitro data can be used to extrapolate that IRAP is useful in treating patients with IDDM. One having ordinary skill in the art following the teachings of this Specification and with a contemporary knowledge in the art, could practice the present invention. Methods of using cytokines as therapeutics are well known. Formulation, handling and administration of cytokines are well known to those having ordinary skill in the art. The method of determining the effective dosage of IRAP for a particular patient is a matter that is within an ordinary level of skill in the art. The Specification enables one having ordinary skill in the art to use the present invention without undue experimentation. By administering IRAP to afflicted patients or those susceptible to IDDM, the IL-1 receptors on insulin producing B-cells can be blocked, thereby preventing IL-1 from binding to the cells. By preventing IL-1 from binding to the IL-1 receptors on the B-cells, the autoimmune mediated damage of pancreatic B-cells caused IL-1 can be averted. Thus, the onset or progress of IDDM can be arrested and normal function of insulin producing cells can be maintained.

To use IRAP as a prophylactic or therapeutic in the prevention or treatment of IDDM, an effective amount of IRAP sufficient to block IL-1 receptors on insulin producing cells is administered to a patient. The amount of IRAP present must be equal to or, more preferably, exceed the amount of IL-1 present. Furthermore, the presence of IRAP must be sustained continually at levels sufficient to effectively compete with the endogenous IL-1. The presence

of IRAP will prevent the damage and death of insulin-producing cells associated with IDDM.

Contemplated equivalents of the present invention include a method of preventing or treating IDDM comprising administration of an effective amount of an IRAP equivalent; an IRAP equivalent being defined as a molecule which is functionally similar and structurally related to IRAP. For example, IRAP equivalents can include: IRAP fragments; molecules with similar amino acids as IRAP but which have some amino acid insertions, deletions or substitutions; and, chimeric proteins containing functional regions of IRAP.

Example 1 Use of IRAP in the Treatment of IDDM

IRAP is formulated either as a solution in saline or buffer at physiological pH, or as an emulsion similar to that used for administration of antibiotics. The purpose of such emulsions is to retard the absorption and the degradation of IRAP. It can also be mixed with adjuvants for the same purpose. IRAP can also be formulated complexed with a human non-neutralizing anti-IRAP antibody to retard degradation and act as a slow release dosage form.

IRAP is used to treat humans shortly or immediately after the diagnosis of IDDM. IRAP is administered at doses ranging from 1 μg/kg to 1000 μg/kg per treatment, and the frequency of treatments varies from once a day to four times a day. The routes of administration include subcutaneous and intramuscular injection intravenous infusion or oral enteric coated preparations. When given several times a day it is preferentially administered orally before meals.

In the preferred embodiment of the present invention, 150 μg/kg/day are administered by intravenous infusion.

IRAP can also be used in humans at high risk of developing IDDM. Thus, first degree relatives of diabetic patients with high titers of ICA and a first phase insulin release below the 5th percentile of the normal population will be treated with IRAP, as described above.

Table 1

	Insulin Release (ng/10 islets)	
IL-1 Exposure	60 min	120 min
Untreated	42.0 ± 4	45 ± 4
IRAP (10000 ng/ml)	44 ± 4	–
IL-1 (10 ng/ml)	8 ± 2*	9 ± 3*
IRAP (10 ng/ml) + IL-1	11 ± 2*	–
IRAP (100 ng/ml) + IL-1	40 ± 4	–
IRAP (1000 ng/ml)	–	41 ± 5
IRAP (1000 ng/ml) + IL-1	44 ± 2	20 ± 4*
IRAP (10000 ng/ml) + IL-1	45 ± 4	–

Results are means ± SEM of 5-6 separate experiments.

* P < 0.001 vs. control (untreated) group, using ANOVA.

Table 2

	Insulin Release (ng/10 islets x 60min)	Insulin Content (ng/10 islets)	DNA Content (ng/10 islets)
Untreated	46 ± 4	528 ± 63	296 ± 12
IRAP (500 ng/ml)	52 ± 11	537 ± 96	262 ± 25
IL-1 (5 ng/ml)	4 ± 1***	348 ± 77*	203 ± 19**
IRAP + IL-1	42 ± 5	441 ± 32	261 ± 16

Results are means ± SEM of 4 separate experiments.

*P<0.02; **P<0.01 and ***P<0.001 vs controls (untreated) group, using paired t test.

Table 3

	RIN m5f cell replication (dmp/μg DNA 60 min)	DNA/dish (μg)
Untreated	4708 $\pm$ 222	89 $\pm$ 3
IRAP (5000 mg/ml)	5211 $\pm$ 456	81 $\pm$ 2
IL-1 (5 ng/ml)	3269 $\pm$ 203*	75 $\pm$ 4*
IRAP + IL-1	4633 $\pm$ 297	91 $\pm$ 4

Results are means $\pm$ SEM of 6 separate experiments.

* P $<$0.015 vs. control (untreated) group, using ANOVA.

. CLAIMS

1. A method for the treatment of insulin dependent diabetes mellitus in humans which comprises administering to a human, who is suffering from said IDDM, an amount of IRAP effective to reduce severity of said IDDM.

2. A method according to Claim 1 where said IRAP is administered by a mode of administration selected from the group consisting of: intravenous infusion; subcutaneous injection; intramuscular injection; and, oral ingestion.

3. A method according to Claim 2 where said IRAP is administered by intravenous infusion.

4. A method according to Claim 1 wherein said amount of IRAP administered to said human is 1 to 1000 μg of IRAP per kilogram of human per day.

5. A method according to Claim 4 wherein said amount of IRAP administered to said human is 100 to 500 μg of IRAP per kilogram of human per day.

6. A method according to Claim 5 wherein said amount of IRAP administered to said human is 150 μg of IRAP per kilogram of human per day.

7. A method of protecting insulin-producing cells from destructive effects of IL-1 which comprises administering an amount of IRAP effective to prevent destruction of said insulin-producing cells by IL-1.

8. Use of IRAP to prepare a medicament for the treatment of insulin dependent diabetes mellitus.

9. Use of IRAP to prepare a medicament for protecting insulin-producing cells from destructive effects of IL-1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/09151

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	Nature, vol. 344, 12 April 1990, (London, GB), D.B. CARTER et al.: "Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein", pages 341-346, see the abstract; page 637, right-hand column, lines 26-44 (cited in the application) ---	1,7-9
Y	FEBS Letters, vol. 261, no. 1, February 1990, (Amsterdam, NL), P. HAMMONDS et al.: "Insulin-secreting beta-cells possess specific receptors for interleukin-1beta", pages 97-100, see the abstract; page 99, right-hand column, line 34 - page 100, left-hand column, line 2 (cited in the application) --- -/-	1,7-9

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11-03-1992	09. 04. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NURIA TORIBIO

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	Diabetologia, vol. 34, no. 6, June 1991, (Berlin, DE), D.L. EIZIRIK et al.: "An interleukin-1 receptor antagonist protein protects insulin-producing beta cells against suppressive effects of interleukin-1beta", pages 445-448, see the whole document (cited in the application) -----	1,7-9

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers 2-6 because they relate to subject matter not required to be searched by this Authority, namely:

see PCT-Rule 39.1(iv)
Remark: Although claims 1,7 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.